Multitest Screening in Hematology*

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The concept of multitest screening for hematological disorders is not necessarily a new one. Implementation of such ideas has recently become possible, for the automated electronic instruments performing sequential multiple analyses within very short periods of time are a very significant advancement in the technology of the hematology laboratory. It must be emphasized, however, that the value of such instrumentation lies not only in the rapidity, but also in the accuracy and precision as well as the number and nature of the tests performed. While the data on samples have been reported on individual patients three and a half years in our hematology laboratory, it is only within the past few months that examples of its utilization for screening studies have begun to appear in the literature (1, 3, 5).

Several automated instruments are available that perform the routine counting and sizing of cells in the blood. The Coulter model "S" is the one most widely used (2). This is the instrument we use and it is the one used in the studies to be cited here. It is an instrument that reports on seven parameters, measuring the WBC, RBC, and mean corpuscular volume (MCV), and utilizing the latter two computing the hematocrit and then the mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The time required from the time of sample aspiration until the results of the seven determinations are printed is 40 seconds. I must emphasize that not only speed is achieved, but precision and accuracy are far greater than many previously used methods. Indeed, variation on replicate samples is less than 1% and accuracy appears to be of the same order.

Considering the availability of such accurate data and the comparative ease with which they may be obtained, it can be seen that these instruments can be valuable tools in screening for hematological abnormalities. Indeed here at the Medical College of Virginia, two interdepartmental cooperative studies have been conducted within the past year. One of these was presented in abstract form at the December 1972 meeting of the American Society of Hematology, and has recently been submitted for definitive publication (5). The work in large part was the project of Mr. Alvin Schmaier, currently a third-year medical student, with assistance from Drs. Maurer, Johnston and Scott. The other study has been conducted as part of a larger study in approximately 530 Black children in the Head Start Program. These studies exemplify particularly well the principle of multitest screening in hematology, and it is to these and to a comparison of these with previous studies (1, 3) that I wish to address myself.

I will not detail the technical operation of the model "S" but note here that we have had the instrument in operation for more than three and a half years. This has given us extensive experience in all of the positive as well as the negative qualities of the instrument. Because some of the parameters are computed, our initial efforts were to compare these with other methods then in operation and standardize the data. This was accomplished by comparative data obtained on 1,500 samples of EDTA anticoagulated blood. No significant difference was found in the hemoglobin determination, but the linearity was better in the model "S" determinations than with flow-through determinations giving greater accuracy to the former, particularly in the high > 16 gm and low < 7 gm ranges. For reasons which have never been satisfactorily explained either by us or by the manufacturer, the WBCs average approximately 8% lower by the model "S" than when determined by our alternative or electronic back-up. This discrepancy is noted also in data from other institutions. The lower percentage has been consistent

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and therefore is not a factor. Finally, the comparison of the spun hematocrit with the computed hematocrit (MCV × RBC) showed the latter to be, on the average, 1% higher—a difference that is minimal and one that certainly can be disregarded. I should note here that this is somewhat different from the study of England and Fraser (1) who set the electronics so that the hematocrit is 1.5% below the spun hematocrit. This quite possibly might allow for differences of our MCV norm 92 ± 6 (range 86–98) from theirs (80–90). Indeed Pearson, *et al.* (3), describe a range of 72–101 with a mean of 88.67 and a standard deviation of 5.3.

At the outset then, it becomes important to note what the norms are for the population being studied and these should be norms for the institution. The data just given are presumed to be an adult population; however, it is not clear from either of the published reports cited above whether age differences were taken into consideration in their studies. The reason for concern with the age variability of the MCV data will become apparent since this parameter is one of the major criteria used to screen for the thalassemia syndromes.

The other measured parameter of extreme importance in the screening efforts is the RBC. We have come to regard this as extremely accurately measured by the model "S." In contrast to the earlier manual methods with an accuracy no greater than 20%, we now feel the accuracy and reproducibility of this measurement is on the order of 1%, or in the range of 50,000 RBC/μl.

With these points in mind, I would like to discuss the application of multitest screening in hematology and share some of the results we have accumulated in specific studies. The first such study undertaken here was a cooperative effort involving the divisions of Clinical Pathology, Pediatrics and Medicine. Mr. Alvin Schmaier, working as a summer extern in the Department of Pediatrics, was responsible for the collection of samples and collation of the data. This portion of my report is in large part his effort. It is a most commendable piece of work which recently has been submitted for publication (5). The data given here are reproduced with his permission.

This study took advantage of the fact that defective alpha chain synthesis may occur in the neonate and result in the presence of the tetrameric hemoglobin, hemoglobin Barts (gamma 4). This form of hemoglobin rapidly disappears with onset of beta chain synthesis and by six months of age no longer can be identified. Cord blood studies have been performed but these naturally are limited in scope. Alpha thalassemia heterozygotes have abnormal red cell indices, and if these could be identified early, it might be possible to select infants in the neonatal period, or certainly within the first few weeks of life, whose blood then could be subjected to hemoglobin electrophoresis.

Taking advantage of the fact that blood could be obtained by capillary puncture with the use of unopettes from Becton Dickinson especially for model "S" counters (order no. 5840, Becton Dickinson, Rutherford, N. J.), Mr. Schmaier began collecting capillary samples in triplicate from the newborn nursery, of course after receiving parental consent. Table 1 shows the tabulation of the results. In particular, I want to call attention to the MCV and MCH. If one were to choose values for identification as suggested by other studies, one would select only those with values below 80 fl! Obviously, then the norm for this population group is quite different from adults, a fact that is well known (6). We were unaware, however, of the limits using the model "S" and these needed definition for the study.

A total of 200 newborn Black infants was studied. Electrophoresis of the hemoglobin in these newborns showed 181 to be normal; that is, only hemoglobins A and F. Of the remainder, 13 showed an additional abnormal hemoglobin either S or C, while six showed the presence of hemoglobin Barts. Of the six, one also showed C and one S hemoglobin. As defined in Table 1, the lower limit of normal was 97 fl. In the samples studied, nine infants had MCV less than 94 fl and MCH less than 29.5 fl. Since these figures are less than normal the data would be "suspect" and thus hemoglobin electrophoresis was performed. In the group of 200 studied, all had hemoglobin electrophoresis performed. Table 2 shows the hemoglobin electrophoresis and pertinent model "S" data on those with abnormal MCV and MCH. It is to be noted that hypochromia can no longer be assessed by the MCHC (4); that is clearly shown in this table. The most striking findings are that of nine patients, six had hemoglobin Barts and thus the alpha thalassemia trait. These were clearly identified by the low MCV and low MCH. Two of the patients had a second abnormality in that Hgb C was identified in one and S in the other. In the total sample, 13 infants were identified who had either

TABLE 1

RED CELL INDICES IN NORMAL BLACK FULL-TERM NEWBORN INFANTS

	Mean	Standard Deviation	ks	Lower Tolerance Limit
Red Blood Cell Count ($\times 10^6/\mu l$)	5.2	0.6	1.0	4.2
Hemoglobin (g/dl)	18.0	2.0	3.3	14.7
Hematocrit (%)	55.3	6.1	10.0	45.3
Mean Corpuscular Volume (fl)	106.4	5.7	9.4	97.0
Mean Corpuscular Hemoglobin (pg)	34.5	2.2	3.6	30.9
Mean Corpuscular Hemoglobin Concentration (g/dl)	32.5	1.0	1.7	30.8

s = Standard Deviation
Constant k = 1.65
Lower tolerance limit = mean-ks
Sample size = 91
For example, for the mean corpuscular volume of 106.4μ^3, the lower limit of normal is 97μ^3 (lower tolerance limit).

Hgb S or C but no Barts and an MCV and MCH within the normal limits. Thus in this study, all infants with alpha thalassemia were readily identified so that no "false negative" values were recorded. There apparently were three false positives, with data just below the norms set. Currently, there is no explanation for these.

It can be seen then that Mr. Schmaier made a significant contribution in the study and that it is possible to screen infants using the multitest principle. As a matter of fact, the incidence of 3% hemoglobin Barts is consistent with the reported incidence in American Blacks.

Let me now turn to another study involving multitest screening with the evaluation of RBC abnormality indices. Whereas the previous study was concerned with alpha thalassemia trait, this study was designed to study thalassemia traits perhaps encompassing both alpha and beta thalassemias. Again, this was a cooperative study involving the same divisions as those in the study just cited.

The group screened was quite different, however. This was composed of 540 Black children most of whom were enrolled in the Head Start Program in Richmond. Our study was only part

TABLE 2

HEMATOLOGIC FINDINGS IN NINE INFANTS WITH MEAN CORPUSCULAR VOLUMES <94 FL AND MEAN CORPUSCULAR HEMOGLOBINS <29.5 PG

Infant	Hgb Pattern	MCV (fl)	MCH (pg)	MCHC (gm/dl)	Hgb Barts (%)	Other Abnormal Hgb (%)
1	F, A, S, Barts	93	28.5	31.0	4.6	4.8
2	F, A, C, Barts	93	28.2	30.7	4.4	10.6
3	F, A, Barts	87	27.7	32.0	5.1	—
4	F, A, Barts	94	29.1	31.4	6.6	—
5	F, A, Barts	93	29.5	32.1	4.8	—
6	A, F, Barts*	90	28.0	31.6	3.0	—
7	F, A	91	29.0	31.2	—	—
8	F, A	93	29.2	31.4	—	—
9	F, A	91	29.3	32.2	—	—

MCV = mean corpuscular volume
MCH = mean corpuscular hemoglobin
MCHC = mean corpuscular hemoglobin concentration
RBC = red blood cell count
Hgb = hemoglobin
Hct = hematocrit
* Electrophoretic pattern at one month of age

of a larger medical evaluation conducted under the aegis of the Department of Pediatrics at the Medical College of Virginia. As part of the study, capillary blood samples were drawn by a registered medical technologist at the various schools. Samples were taken for Coulter model "S" determinations and hemoglobin electrophoresis. As in the earlier study, unopettes for the model "S" (order no. 5840, Becton Dickinson, Rutherford, N. J.) were used. These were drawn in duplicate, the model "S" parameters determined on both samples. Capillary samples also were used for the hemoglobin electrophoresis. Indices available for comparison were dated prior to the advent of electronic counters performing multiple simultaneous tests. Our experience with initial standardization of our model "S" showed that our norm for the automated procedures of 92 ± 6 was higher than that generally used of 88 ± 8. It seemed likely, therefore, that these older data could represent values lower than might be obtained by the currently used automated methods. Additionally, other than the standards cited by Shumway (6), I am aware of no others. As a result, it seemed preferable to determine our own mean model "S" parameters for this age group.

As noted, the recently published works did not specify the ages of the patients studied. It was assumed, therefore, that these were adults. In both studies, the lower limit of normal was 80. With Shumway's data (6) suggesting a lower mean MCV for pediatric age groups when compared with adults, we felt that perhaps it would be wise to include lower values. Thus for those studies, an MCV of 76 or greater was used for establishment of the mean and those 75 or lower were excluded. It is gratifying to note that 26% of the children studied had MCVs 76-79 and would have been excluded if the criteria of other screening studies had been used.

For the moment, I would like to refer to the results not as normal but rather as "mean model 'S' parameters." The health of the children was not evaluated and the sampling was random. Data from two children were excluded because of hemoglobin values less than 10 but with MCV over 80.

As a result, determination on 451 Black children, ages four to eight, with MCV greater than 76 were available for analysis. The results of these determinations are shown in Table 3. As noted, these are mean model "S" parameters for the group studied. These show the means with one standard deviation as well as the range. Also shown is the lower tolerance limit using a 95% tolerance interval (k = 1.65). With these figures we could be 95% certain that 90% of the values would be above the lower tolerance limit shown in the right hand column.

TABLE 3

MEAN MODEL "S" PARAMETERS
(451 Black Children MCV > 75)

Determination	Mean ± SD	Range	Lower Tol. Limit*
RBC	4.51 ± 0.31	3.81–5.19	4.0
Hgb	12.3 ± 0.86	10.4–14.8	10.9
Hct	36.7 ± 2.4	31.1–45.1	32.7
MCV	81.8 ± 3.3	76.0–91.0	76.4
MCH	27.3 ± 1.3	24.3–31.3	25.2
MCHC	33.7 ± 0.8	31.9–35.7	32.4

* Lower Tolerance Limit = $\bar{x} - ks$
$\bar{x}$ = mean
k = 1.65 (95% tolerance level)
s = standard deviation

It now becomes of great interest to compare these results with those alluded to earlier as having been determined by nonautomated methods and reported by Shumway (6). This comparison is shown in Table 4. The similarities are remarkable, especially when one considers that our population sample was not a selected one and was from children with Hgb greater than 10.4 gms and MCV 76 or greater. I think we can conclude that it might be reasonable to accept these as normal values for the age group.

Our original intent for the determination of the indices was to see if it would be possible to screen for thalassemia trait using microcytic hypochromic indices as the major criteria. The spectre of iron deficiency, however, loomed large in front of us since both thalassemia trait and iron de-

TABLE 4

	Current Data	Shumway (6)
RBC	4.51 ± 0.31	4.65 ± .5
Hgb	12.3 ± 0.86	12.7 ± 1
Hct	36.7 ± 2.4	37.0 ± 3
MCV	81.8 ± 3.3	80.0 ± 4
MCH	27.3 ± 1.3	27.0 ± 2
MCHC	33.7 ± 0.8	34.0 ± 1

ficiency are characterized by microcytic hypochromic indices. At the time that these data were being collated for presentation, the results of the hemoglobin electrophoresis were not available. Thus, there were 84 children with MCV 75 or less who needed to be differentiated if possible. Pearson, *et al.* (3), in their studies, proposed a scheme for thalassemia trait screening. This is shown in a slightly modified fashion in figure 1. Note that the first step in screening is to classify the subjects into two groups according to the MCV which is precisely what we did. The next step involves quantification of A_2 hemoglobin which, as noted, is not at the moment available.

This led to a search for an alternative method for attempting to differentiate the MCV 75 and below group. Recently, such a method has been published by England and Fraser (1). They reported that by the use of the statistical discriminant function (D.F.) they were able to differentiate between the disorders with a 99% success rate in 72 cases. As a result of their study, a slightly modified formulation is available and is shown as well as the data for the study in Table 5. Thus, it seemed that it might be possible to predict, in advance of the receipt of the electrophoretic data, the groups into which our 84 subjects might fall and effectively separate the thalassemia trait from an iron deficiency. Accordingly, the appropriate data on those children with MCV 75 or less were substituted into the formula. The results are shown along with the normal in Table 5. It can be seen that 11 of the group had negative, while 73 had positive, D.F.s. To show the distribution, a chart like that of England and Fraser (1) was prepared and is shown in figure 2. The closed circles represent those subjects with a positive D.F.′ and presumable iron deficiency. Those with the open circles represent the negative D.F.′ and presumable thalassemia trait. Since the critical data currently are missing, I think it is only possible to state that we are predicting that these are the groups into which the subjects fall. The data in Table 5 show certain of the mean values to be sufficiently different to suggest that these really are two distinct groups despite a few (four) that were too close to 0 to call (fig. 2). As soon as the additional data are available, we will know how well we are able to predict.

If I may be allowed to speculate, I expect a high success rate. As evidence for this rather rash statement, again direct your attention to figure 2. Note that in the D.F.′ negative area [D.F.′ = MCV −RBC − (5 × Hgb) − 3.4] there are four x's. While this manuscript was in preparation, I noted four patients' model "S" data that were quite like those seen in the 11 children with negative D.F.s. I felt these were suspect on the basis

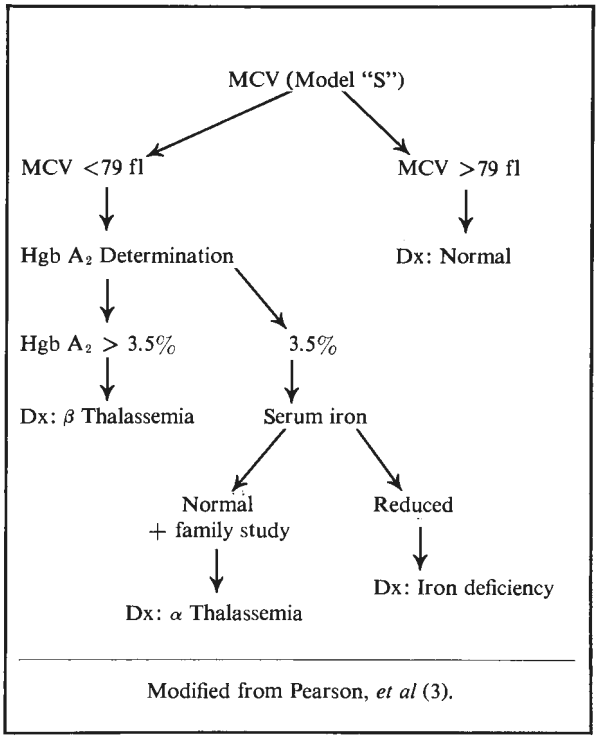

Fig. 1—Suggested schema for thalassemia trait screening.

TABLE 5

COMPARISON OF GROUPS BY MCV AND D.F.′*

# of Children	451	11	73
		MCV < 76	
	MCV > 75	D.F.′−	D.F.′+
RBC	4.51 ± 0.31	*5.54 ± 0.19	4.86 ± 0.28
Hgb	12.3 ± 0.86	12.5 ± 0.67	11.6 ± 0.70
Hct	36.7 ± 2.4	37.7 ± 1.79	35.2 ± 2.14
MCV	81.8 ± 3.3	*68.4 ± 3.8	*72.9 ± 2.57
MCH	27.3 ± 1.3	*22.6 ± 1.75	*24.0 ± 1.15
MCHC	33.7 ± 0.8	33.3 ± 1.03	33.1 ± 0.93

* D.F.′ = MCV − RBC − (5 × Hgb) − 3.4

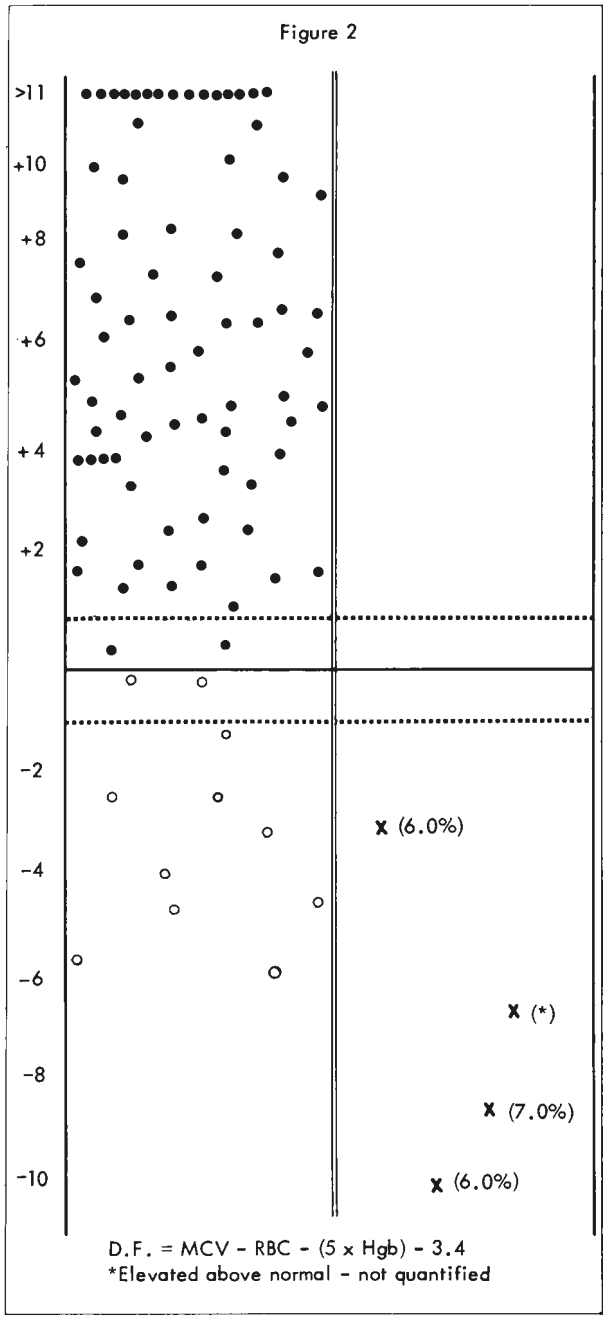

Fig. 2

of the experience I was accumulating. Hemoglobin electrophoresis was performed in these, therefore, and the A_2 hemoglobin quantified. Summary data on these patients are given in Table 6. Such ability to predict certainly suggests that the automated multitest apparatus has given us remarkable ability

TABLE 6

Patient	RBC	MCV	MCH	D.F.	% A_2Hgb
D.H.	5.65	62	20	−3.05	6
W.F.	6.52	64	20	−9.92	6
A.J.	6.17	65	21	−8.5	7
P.F.	6.16	64	20	−6.5	(*)

* Elevated above normal—not quantified

to select those most likely to have beta thalassemia trait. I await with eagerness complete collation of data, predictions and electrophoresis in the 84 children noted.

In summary, it can be said that a sample of multitest screening in hematology has been presented, made possible by the use of automated counting apparatus. The ease, precision and accuracy of the determinations favor much wider application. To this end studies are now cropping up in the literature, almost with every new journal that hits one's desk. Our efforts presented here show that studies of "at risk" populations are not only feasible but practical. Such examples as those given, certainly advance our knowledge and facilitate our know-how in difficult diagnostic areas.

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